ORIGINAL ARTICLE 10.1111/j.1469-0691.2004.00931.x

Serotypes and clinical manifestations of invasive group B streptococcal infections in western Sweden 1998–2001

E. Persson[1], S. Berg[1], B. Trollfors[1], P. Larsson[2], E. Ek[2], E. Backhaus[3], B. E. B. Claesson[3], L. Jonsson[4], G. Rådberg[5], T. Ripa[6] and S. Johansson[6]

[1]Department of Paediatrics, The Queen Silvia Children's Hospital, [2]Department of Bacteriology, Sahlgrenska University Hospital, Göteborg, [3]Central Hospital of Skövde, Skövde, [4]Borås Hospital, Borås, [5]Uddevalla Hospital, Uddevalla and [6]Halmstad Hospital, Halmstad, Sweden

ABSTRACT

This study monitored the serotypes of *Streptococcus agalactiae* (group B streptococcus; GBS) isolated from invasive infections in western Sweden and investigated possible relationships between serotype, age and clinical manifestations. Invasive GBS isolates were collected prospectively during 1998–2001 at six laboratories, covering two counties with a population of 1.8 million, and were serotyped by coagglutination. Clinical data were obtained from hospital notes. In total, 161 invasive strains (50 from neonates and infants aged <3 months, and 111 from adults) were serotyped. The commonest serotypes from neonates and infants were serotypes III (60%), V (22%) and Ia (10%), and from adults were serotypes V (42%) and III (25%). Serotype V had doubled in frequency among both children and adults compared to a previous study from the same area in 1988–1997. Most (80%) of the adults had an underlying medical condition. No relationship was found between serotype and clinical manifestations. However, the study demonstrated the importance of active surveillance of GBS serotypes and the difficulties of formulating a multivalent polysaccharide conjugate vaccine against GBS.

Keywords Epidemiology, group B streptococcus, neonatal, serotypes, *Streptococcus agalactiae*

Original Submission: 7 September 2003; **Revised Submission:** 30 November 2003; **Accepted:** 29 February 2004

Clin Microbiol Infect 2004; 10: 791–796

INTRODUCTION

Streptococcus agalactiae (group B streptococcus; GBS) is a leading cause of invasive neonatal infections and infections in pregnant women. In non-pregnant adults, GBS infection has also become an increasingly common cause of invasive disease, particularly in elderly persons and among those with underlying medical conditions [1–3]. A combination of host and microbial factors determines the outcome in infected individuals [4]. Based on the composition of the capsular polysaccharide, group B streptococci can be divided currently into nine different serotypes. The prevalence of different serotypes varies according to time and geographical location [5–12]. Serotypes Ia, Ib, II and III have predominated in many parts of the world [6,12], but serotype V has emerged as an increasingly important pathogen [5,7]. Serum antibodies against the capsular polysaccharide provide type-specific protection against invasive GBS infections [13–16]. The high morbidity and mortality of invasive GBS infections has made the development of a multivalent conjugate polysaccharide vaccine a major focus for research [17–23]. Therefore, knowledge of the distribution and changes in GBS serotypes in different populations is important.

The main aim of the present study was to survey the serotype distribution of invasive GBS isolates in a Swedish population and to detect changes in serotype distribution over time. A second objective was to determine whether the

Corresponding author and reprint requests: E. Persson, Department of Paediatrics, Sahlgrenska University Hospital, The Queen Silvia Children's Hospital, S-416 85 Göteborg, Sweden
E-mail: elisabet.persson@vgregion.se

serotype distribution differed between age groups and patients with different clinical manifestations.

MATERIALS AND METHODS

Population-based surveillance

Invasive GBS isolates were collected prospectively from the six bacteriological laboratories in the counties of Västra Götaland and Halland in western Sweden between 1998 and 2001. These laboratories served all 13 hospitals in the two counties. The mean population of the surveillance area was 1 767 215, and the total number of live births was 72 641 during the study period (data obtained from the Central Bureau of Statistics, Stockholm, Sweden; http://www.scb.se). GBS isolates were collected from normally sterile sites (blood, cerebrospinal fluid and synovial fluid). Isolates were identified as GBS by colony morphology, microscopy following Gram's stain of smears, and coagglutination with group-specific reagents (Streptex; Murex Biotech, Dartford, UK). The isolates were stored in broth at −70°C until serotyping was performed. Only one isolate from each infectious episode was included in the study. Clinical data (age, sex, gestational age, underlying medical conditions, clinical manifestations and outcome) were obtained from individual hospital notes. All hospital notes and relevant data were available for all patients. The policy for intrapartum antimicrobial prophylaxis in the region was the same as that now recommended by the American College of Obstetricians and Gynecologists [24]. The study was approved by the Ethics Committees of Göteborg University and Lund University.

Serotyping

GBS isolates were serotyped by coagglutination (ESSUM Group B Streptococcus Serotyping Test; Bacterum AB, Umeå, Sweden) with type-specific antisera for serotypes Ia, Ib, II, III, IV, V, VI, VII and VIII [25]. The only isolate not typeable by coagglutination was examined with precipitation techniques (ring test and diffusion test) [26,27] for serotypes I–VIII by J. Motlová, National Streptococcus and Enterococcus Reference Laboratory, National Institute of Public Health, Prague, Czech Republic. Differences in serotype distribution were compared with chi-squared tests (2×7 tables).

RESULTS

During the period 1998–2001, 165 patients with 167 episodes of invasive GBS disease (annual incidence of 2.4 cases/100 000 inhabitants) were recorded. In total, 161 invasive GBS isolates were available for serotyping. Fifty (31%) of these isolates were obtained from neonates and infants, and 111 (69%) from adults. No patient with invasive GBS infection was found in the group aged 3 months to 18 years. Isolates from six patients (two neonates and four adults) were lost from one of the laboratories.

Neonates and infants

During the 4-year period, 52 invasive GBS infections in neonates and infants aged 0–86 days (33 boys, 19 girls) were documented. Of these cases, 42 (81%) were early onset (< 7 days after birth), eight were late onset (7–27 days after birth), and two were very late onset (28 days to 4 months after birth). The incidence in neonates aged <28 days was 0.6/1000 live births, and in neonates and infants aged <4 months it was 0.7/1000 live births.

Infection without a known focus was the commonest manifestation (37 cases, 71%). Seven (13%) patients had meningitis (incidence 0.1/1000), while six patients had pneumonia and two had septic arthritis. In the early onset group, 32 (76%) of the infections appeared as sepsis without a focus, six were pneumonia, and four were meningitis. In the late and very late onset group, four of the infections were sepsis without a focus, four were meningitis, and two were arthritis. The serotype distribution compared to clinical manifestations in neonates is shown in Fig. 1.

Eleven (21%) patients were pre-term, i.e., <37 weeks of gestational age. Eight of the preterm children had very early onset disease and, of those, seven had other risk factors for infection (i.e., mothers with infection or prolonged rupture of the membrane). Four of the 52 neonates died. Two died following early onset disease (sepsis without a focus), and two following late onset disease (meningitis and sepsis without a focus, respectively).

Fifty of the GBS isolates from neonates and infants were available for typing (Table 1). The

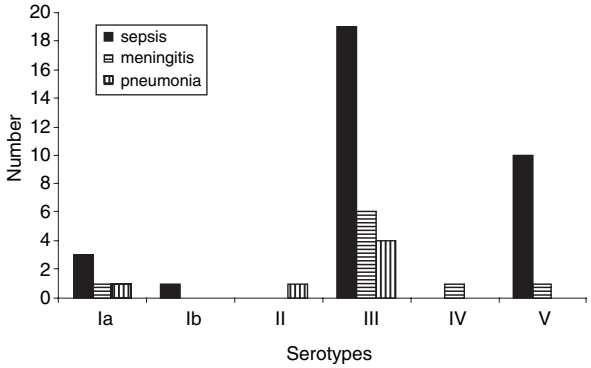

Fig. 1. Serotype distribution among neonates with different clinical manisfestations.

Table 1. Serotype distribution among invasive isolates of group B streptococci

	Neonates and infants					
	Early onset		Late and very late onset		Adults	
Serotype	No.	(%)	No.	(%)	No.	(%)
Ia	5	(13)	0	(0)	10	(9)
Ib	1	(3)	1	(10)	10	(9)
II	1	(3)	0	(0)	7	(6)
III	24	(60)	6	(60)	28	(25)
IV	0	(0)	1	(10)	8	(7)
V	9	(23)	2	(20)	47	(42)
Non-typeable	0	(0)	0	(0)	1	(1)
Total	40		10		111	

Table 2. Clinical manifestations and serotype distribution of invasive group B streptococci in adults

	No. of patients							
Manifestation	Ia	Ib	II	III	IV	V	NT	Total
Sepsis, unknown focus	6	1	4	11	3	17	0	42
Erysipelas	1	2	2	4	1	6	1	17
Endocarditis	1	2	0	3	2	3	0	11
Endometritis	0	3	0	1	1	5	0	10
Meningitis	0	0	0	1	0	1	0	2
Arthritis	0	0	0	2	0	7	0	9
Urosepsis	0	1	0	2	0	3	0	6
Other	2	1	1	4	1	5	0	14
Total	10	10	7	28	8	47	1	111

commonest serotypes were serotypes III (60%) and V (22%). There were no significant differences related to post-natal age (Table 1), manifestation (Fig. 1), gestational age, hospital or outcome (details not shown).

Adults

In adults aged ≥18 years, 115 invasive GBS infections were identified. The incidence was 2.1/100 000 inhabitants/year. The median age of the adults (55 males, 60 females) was 68 years (range 19–96 years). In total, 111 isolates were available for typing (Table 1). Serotypes III and V were the commonest serotypes (25% and 42%, respectively). Serotypes Ia, Ib, II and IV were isolated at similar frequencies (6–9%). The serotype distribution related to clinical manifestations is shown in Table 2. Ninety-four (82%) patients had a known underlying medical condition, with the commonest being diabetes mellitus (n = 30) and malignant disease (n = 26). Ten (9%) patients were pregnant. Ten (9%) patients died during or soon after the infection. The serotypes isolated from these latter ten patients were Ia (four), Ib (two), III (two), IV (one) and non-typeable (one).

Table 3. Serotype distribution of invasive group B streptococci isolates in the present study compared with a previous study in the same region

	Neonates				Adults			
	Berg *et al.* [12]		Present study		Berg *et al.* [12]		Present study	
Serotype	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Ia	14	(18)	5	(10)	4	(6)	10	(9)
Ib	2	(3)	2	(4)	15	(23)	10	(9)
II	4	(5)	1	(2)	10	(15)	7	(6)
III	48	(62)	30	(60)	19	(29)	28	(25)
IV	2	(3)	1	(2)	1	(2)	8	(7)
V	7	(9)	11	(22)	14	(21)	47	(42)
Non-typeable	1	(1)	0	(0)	3	(5)	1	(1)

Two patients had two infectious episodes at intervals of 7 and 3.5 months. The recurrent infections were caused by the same serotypes as the initial infections in both patients (serotypes II and V, respectively). The manifestations were erysipelas and septic arthritis, respectively. One patient had Addison's disease, while the other patient had a rheumatic disease and bilateral knee prostheses.

A significant difference in the serotype distribution between adults and neonates was found ($p < 0.002$; Table 1), associated chiefly with the occurrence of serotypes III and V. Table 3 compares the serotyping results with those obtained in a previous study in the same region [12].

DISCUSSION

In the present study, the annual incidence of GBS infection was 2.4/100 000 inhabitants, which was almost identical to that found in a previous survey of a part of the same area [28]. The incidence among neonates and infants was also similar to that found in other studies of neonatal infections from the Göteborg area in western Sweden, in which the incidence rates were 0.8 and 1.0/1000 [29,30]. The present data were also consistent with results from other studies in Europe and North America [6,10,31–33]. However, comparison with a previous study in the same region of strains isolated in 1988–1997 [12] showed significant changes in the serotype distribution of GBS among strains from both neonates ($p < 0.0001$) and adults ($p < 0.0001$). The most important change in both groups was a pronounced increase in infections caused by serotype V. This increase was not paralleled by a decrease in a single serotype, but there were

smaller decreases in the proportions of several other serotypes. Serotype V was first reported in 1985 [34], and is now the commonest serotype among adults in North America, Taiwan and Zimbabwe [7,8,10,35], as well as in the present study. Recent studies from Canada and Taiwan [6,35] also show the importance of GBS type V in neonates, although serotype III has been, and remains, the commonest serotype in Europe and North America [6,10,12,31,33,36]. Type III was also the second most common serotype in adults in the present study, and has been the second or third most common serotype in adults in other recent studies [5,7,10]. Compared to serotypes III and V, all other serotypes were uncommon, and only type Ia has been more important in a few studies [5,37]. In the present study, serotype Ia accounted for 10% of the neonatal cases and 9% of the adult cases.

It is important to note that non-typeable strains, which are probably non-capsulated, have been isolated from patients with invasive infections, even though the capsule is of major importance for protection of bacteria against phagocytosis. In previous studies, the proportion of non-typeable isolates in invasive infections has varied from 1% to 10% [10,12,31,37].

The most important source of GBS to which the neonate is exposed is the maternal genital tract. Approximately 15–25% of pregnant women are colonised with GBS in the urethra, cervix and/or rectum [36,38]. The serotype distribution of colonising strains is similar to the distribution of invasive strains, but two studies have shown that the proportion of serotype III strains is higher among invasive strains than among colonising strains [12,36]. This indicates that serotype III strains may be more virulent than strains of other serotypes, which could be related to a failure to mount an adequate serum antibody response to serotype III during colonisation. There are some unexplained geographical differences between the serotype distributions of colonising GBS strains. The most striking is the high prevalence of serotypes VI and VIII among pregnant Japanese women [11], while these strains seem currently to be non-existent or rare in Europe and North America [5,12,36].

The nine capsular polysaccharides found in GBS are immunologically distinct, and protection against invasive infection is achieved by serotype-specific circulating serum antibodies [13–15]. Second to exposure to the organism, the most important protective factor against invasive infection in the neonate is probably the concentration of serum IgG antibodies to the colonising strain(s) in the mother [13–16], particularly as IgG is transferred from the mother to the foetus towards the end of the pregnancy. This forms the basis for efforts to develop GBS vaccines composed of relevant GBS capsular polysaccharides. According to the present study, an effective conjugate vaccine with capsular polysaccharide of GBS serotypes Ia, III and V would, theoretically, provide protection for 84% of all neonatal and infant cases with a gestational age of >34 weeks, and for 76% of all adult cases, in western Sweden. An experimental multivalent conjugate vaccine containing serotypes I–III would, theoretically, prevent 80% of all neonatal and infant cases with a gestational age of >34 weeks, and 57% of all adult cases, in western Sweden [22].

GBS strains also produce one or more strain-variable and surface-localised proteins. These include the c^{α} protein [39,40], the R1–R4 proteins [41,42], the Rib protein [43] and the Sip protein [44]. Studies from Canada have demonstrated that the Sip protein is produced by all GBS isolates, and that vaccination with recombinant Sip protein protects adult mice against experimental GBS infection [44,45].

In conclusion, the pronounced changes in the serotype distribution of GBS strains that have occurred in western Sweden during a relatively short period of time demonstrate the difficulty in formulating a capsule-based GBS vaccine. Alternative strategies for prevention of GBS infections should be considered, e.g., vaccines based on GBS surface proteins that are common to all or most GBS strains.

ACKNOWLEDGEMENTS

Support in the form of grants was received from Gothenburg Medical Association, the Faculty of Medicine, Göteborg University, and the Regional Health Care Authority of Western Sweden. We are grateful to J. Motlová for serotyping the strain that was not typeable by coagglutination, and to the staff at the bacteriological laboratory at Sahlgrenska University Hospital/East.

REFERENCES

1. Bolanos M, Canas A, Santana OE, Perez-Arellano JL, de Miguel I, Martin-Sanchez AM. Invasive group B strepto-

coccal disease in nonpregnant adults. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 837–839.
2. Farley MM. Group B streptococcal disease in nonpregnant adults. *Clin Infect Dis* 2001; **33**: 556–561.
3. Schwartz B, Schuchat A, Oxtoby MJ, Cochi SL, Hightower A, Broome CV. Invasive group B streptococcal disease in adults. A population-based study in metropolitan Atlanta. *JAMA* 1991; **266**: 1112–1114.
4. Schuchat A. Epidemiology of group B streptococcal disease in the United States: shifting paradigms. *Clin Microbiol Rev* 1998; **11**: 497–513.
5. Blumberg HM, Stephens DS, Modansky M *et al.* Invasive group B streptococcal disease: the emergence of serotype V. *J Infect Dis* 1996; **173**: 365–373.
6. Davies HD, Raj S, Adair C, Robinson J, McGeer A. Population-based active surveillance for neonatal group B streptococcal infections in Alberta, Canada: implications for vaccine formulation. *Pediatr Infect Dis J* 2000; **20**: 879–884.
7. Tyrrell GJ, Senzilet LD, Spika JS *et al.* Invasive disease due to group B streptococcal infection in adults: results from a Canadian, population-based, active laboratory surveillance study—1996. Sentinel Health Unit Surveillance System Site Coordinators. *J Infect Dis* 2000; **182**: 168–173.
8. Moyo SR, Maeland JA, Bergh K. Typing of human isolates of *Streptococcus agalactiae* (group B streptococcus, GBS) strains from Zimbabwe. *J Med Microbiol* 2002; **5**: 595–600.
9. Farley MM, Harvey RC, Stull T *et al.* A population-based assessment of invasive disease due to group B streptococcus in nonpregnant adults. *N Engl J Med* 1993; **328**: 1807–1811.
10. Harrison LH, Elliott J, Dwyer DM *et al.* Serotype distribution of invasive group B streptococcal isolates in Maryland: implications for vaccine formulation. *J Infect Dis* 1998; **177**: 998–1002.
11. Lachenauer CS, Kasper DL, Shimada J *et al.* Serotypes VI and VIII predominate among group B streptococci isolated from pregnant Japanese women. *J Infect Dis* 1999; **179**: 1030–1033.
12. Berg S, Trollfors B, Lagergard T, Zackrisson G, Claesson BA. Serotypes and clinical manifestations of group B streptococcal infections in western Sweden. *Clin Microbiol Infect* 2000; **6**: 9–13.
13. Baker CJ, Kasper DL. Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection. *N Engl J Med* 1976; **294**: 753–756.
14. Klegerman ME, Boyer KM, Papierniak CK, Levine L, Gotoff SP. Type-specific capsular antigen is associated with virulence in late-onset group B streptococcal type III disease. *Infect Immun* 1984; **44**: 124–129.
15. Lin FY, Philips JB, Azimi PH *et al.* Level of maternal antibody required to protect neonates against early-onset disease caused by group B streptococcus type Ia: a multicenter, seroepidemiology study. *J Infect Dis* 2001; **184**: 1022–1028.
16. Baker CJ, Kasper DL, Tager IRAB *et al.* Quantitative determination of antibody to capsular polysaccharide in infection with type III strains of group B streptococcus. *J Clin Invest* 1977; **59**: 810–818.
17. Kasper DL, Paoletti LC, Wessels MR *et al.* Immune response to type III group B streptococcal polysaccharide–tetanus toxoid conjugate vaccine. *J Clin Invest* 1996; **98**: 2308–2314.
18. Baker CJ, Rench MA, Edwards MS, Carpenter RJ, Hays BM, Kasper DL. Immunization of pregnant women with a polysaccharide vaccine of group B streptococcus. *N Engl J Med* 1988; **319**: 1180–1185.
19. Baker CJ, Paoletti LC, Wessels MR *et al.* Safety and immunogenicity of capsular polysaccharide–tetanus toxoid conjugate vaccines for group B streptococcal types Ia and Ib. *J Infect Dis* 1999; **179**: 142–150.
20. Paoletti LC, Kasper DL. Conjugate vaccines against group B streptococcus types IV and VII. *J Infect Dis* 2002; **186**: 123–126.
21. Paoletti LC, Peterson DL, Legmann R, Collier RJ. Preclinical evaluation of group B streptococcal polysaccharide conjugate vaccines prepared with a modified diphtheria toxin and a recombinant duck hepatitis B core antigen. *Vaccine* 2001; **20**: 370–376.
22. Kotloff KL, Fattom A, Basham L, Hawwari A, Harkonen S, Edelman R. Safety and immunogenicity of a tetravalent group B streptococcal polysaccharide vaccine in healthy adults. *Vaccine* 1996; **14**: 446–450.
23. Paoletti LC. Potency of clinical group B streptococcal conjugate vaccines. *Vaccine* 2001; **19**: 2118–2126.
24. American College of Obstetricians and Gynecologists. ACOG Committee Options 279. Prevention of early-onset Group B streptococcal disease in newborns. *Obstet Gynecol* 2002; **100**: 1405–1412.
25. Håkansson S, Burman LG, Henrichsen J, Holm SE. Novel coagglutination method for serotyping group B streptococci. *J Clin Microbiol* 1992; **30**: 3268–3269.
26. Williams R. Laboratory diagnosis of streptococcal infections. *Bull WHO* 1958; **19**: 153–176.
27. Rotta J, Krause RM, Lancefield RC, Everly W, Lackland H. New approaches for the laboratory recognition of M types of group A streptococci. *J Exp Med* 1971; **134**: 1298–1315.
28. Dahl M, Tessin I, Trollfors B. Invasive group B streptococcal infections in Sweden: incidence, predisposing factors and prognosis. *Int J Infect Dis* 2003; **7**: 113–119.
29. Tessin I, Trollfors B, Thiringer K. Incidence and etiology of neonatal septicaemia and meningitis in Western Sweden 1975–86. *Acta Paediatr Scand* 1990; **79**: 1023–1030.
30. Persson E, Trollfors B, Lind Brandberg L, Tessin I. Septicaemia and meningitis in neonates and during early infancy in the Göteborg area of Sweden. *Acta Paediatr* 2002; **91**: 1087–1092.
31. Kalliola S, Vuopio-Varkila J, Takala A, Eskola J. Neonatal group B streptococcal disease in Finland: a ten-year nationwide study. *Pediatr Infect Dis J* 1999; **18**: 806–810.
32. Schrag S, Zywicki S, Farley M *et al.* Streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med* 2000; **342**: 15–20.
33. Hervás J, Gonzáles L, Gil J, Paoletti L, Madoff C, Benedí V. Neonatal group B streptococcal infection in Mallorca, Spain. *Clin Infect Dis* 1993; **16**: 714–718.
34. Jelinkova J, Motlova J. Worldwide distribution of two new serotypes of group B streptococci: type IV and provisional type V. *J Clin Microbiol* 1985; **21**: 361–362.
35. Ko WC, Lee HC, Wang LR, Lee CT, Liu AJ, Wu JJ. Serotyping and antimicrobial susceptibility of group B streptococcus over an eight-year period in southern Taiwan. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 334–339.
36. Davies D, Adair C, McGeer D *et al.* Antibodies to capsular polysaccharides of group B streptococcus in pregnant

Canadian women: relationship to colonization status and infection in the neonate. *J Infect Dis* 2001; **184**: 285–291.
37. Lin FY, Clemens JD, Azimi PH *et al*. Capsular polysaccharide types of group B streptococcal isolates from neonates with early onset systemic infection. *J Infect Dis* 1998; **177**: 790–792.
38. Campell J, Hillier S, Krohn M, Ferrieri P, Zaleznik D, Baker CJ. Group B streptococcal colonization and serotype-specific immunity in pregnant women at delivery. *Obstet Gynecol* 2000; **96**: 498–503.
39. Bevanger L, Iversen O-J, Naess AI. Characterization of the α-antigen of the c proteins of group B streptococci (GBS) using a murine monoclonal antibody. *APMIS* 1995; **103**: 731–736.
40. Bevanger L. The Ibc proteins of group B streptococci: isolation of the α and β antigens by immunosorbent chromatography and test for human serum antibodies against the two antigens. *Acta Pathol Microbiol Immunol Scand B* 1985; **93**: 113–119.
41. Lancefield RC, Perlmann GE. Preparation and properties of a protein (R antigen) occurring in streptococci of group A type 28 and in certain streptococci of other serological groups. *J Exp Med* 1952; **96**: 83–97.
42. Bevanger L, Kvam AI, Maeland JA. A *Streptococcus agalactiae* R protein analyzed by polyclonal and monoclonal antibodies. *APMIS* 1995; **103**: 731–736.
43. Stålhammar-Carlemalm M, Stenberg L, Lindahl G. Protein Rib: a novel group B streptoccocal cell surface protein that confers protective immunity and is expressed by most strains causing invasive infection. *J Exp Med* 1993; **177**: 1593–1603.
44. Brodeur B, Boyer M, Charlebois I *et al*. Identification of group B streptococcal Sip protein, which elicits cross-protective immunity. *Infect Immun* 2000; **68**: 5610–5618.
45. Martin D, Rioux S, Gagnon E *et al*. Protection from group B streptococcal infection in neonatal mice by maternal immunization with recombinant Sip protein. *Infect Immun* 2002; **70**: 4897–4901.